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PRINCIPAL INVESTIGATOR: Leslie C. Baxter, PhD

CONTRACTING ORGANIZATION: St. Joseph's Hospital and Medical Center

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Leslie C. Baxter, PhD

email: leslie.baxter@dignityhealth.org

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St. Joseph's Hospital and Medical Center
350 West Thomas Road
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14. ABSTRACT

Purpose: As the first diagnosed ASD individuals are now reaching old age, it is imperative that we understand the impact of aging on individuals with ASD. We developed a model predicting greater executive dysfunction and frontal lobe susceptibility in ASD beyond normal aging. Scope: This study, which is a collaborative study of the Southwest Autism Research and Resource Center and the Barrow Neurological Institute, produces comprehensive cognitive, behavioral, and neuroimaging data on a set of well-characterized older ASD individuals who can be used as a reference for clinical diagnosis, therapeutics, and care plans. The **scope** of the first year was to obtain institutional and HRPO approval to perform the study, to obtain data on a new cohort of older ASD individuals and controls, and analyze and present initial data at a national conference. **Results and significance:** We obtained all regulatory approvals and are recruiting new participants. Current analyses on cross-sectional data found that the older ASD group performed significantly worse in executive functioning and showed decreased white matter connectivity in the frontal and temporal lobes. These data were presented

15. SUBJECT TERMS

Autism, aging, functional MRI, fMRI, neuroimaging, executive functioning, memory, cognition, cortical thickness, connectivity, white matter, sparse Bayesian networks, machine learning

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1. **INTRODUCTION:**

As the first diagnosed Autism Spectrum Disorder (ASD) individuals are now reaching old age, it is imperative that we understand the impact of aging on individuals with ASD. Given the striking parallels in ASD of deficits in executive function, subserved by the frontal lobe, and that the frontal lobe is susceptible to normal age-related changes, we combine neuroimaging, cognitive assessments and behavioral measures to examine aging in ASD compared to Typically Developed (TD) adults. We **hypothesize** that individuals with ASD will have an exacerbation of deficits beyond normal aging, as evidenced in significantly lower scores on tests affected by aging (e.g., executive) along with neuroanatomical markers of dysfunction, and relative preservation of function subserved by more posterior brain regions (memory and local detail processing). Our **objective** is to produce comprehensive cognitive, behavioral, and neuroimaging data on a group of well-characterized older individuals with ASD who can be used as a reference for clinical diagnosis, therapeutics, and care plans. To achieve this goal, our three year project involves longitudinal assessment of aging (40–60 y.o.) ASD individuals versus age-matched TD. In addition to commonly used statistical methods, we will use innovative machine learning and sparse Bayesian networks to combine structure, function, cognition, and symptom profiles to specifically address contributions to accelerated aging in ASD.

2. **KEYWORDS:** Autism, aging, functional MRI, fMRI, neuroimaging, executive functioning, cognition, memory, white matter, cortical thickness, connectivity

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

The major goals for the project in the first year of funding were:

A) **To obtain institutional IRB at the two participating institutions, Southwest Autism Resource and Research Institute (SARRC), involved in participant recruitment and confirmation of Autism diagnosis, and Barrow Neurological Institute (BNI), involved in data acquisition and analysis.** Approval was estimated to take place in the first three months of Year 1. The process required obtaining separate institutional approval from both participating sites followed by HRPO approval. This process was somewhat slower than expected, and was completed in January 2015, which is 3 months past our projected completion of this goal.

B) **Begin recruitment of ASD and age-matched controls.** In our original Statement of Work, recruitment was scheduled for 3-18 months. Recruitment is somewhat behind our anticipated schedule. This was due in part to the delay in obtaining institutional approval. Recruitment has been somewhat slow due some loss to follow-up of some potential ASD participants from the SARRC pool. Since obtaining all of the institutional approvals, we have been involved in a number of community events and this is increasing the rate of new recruits. At the time of this writing, we have 21 older ASD participants and 19 older controls of the 35 needed per each group.

C) **To obtain cognitive and MRI data from ASD and control participants.** We have all procedures for 38/40 of the enrolled participants. Two ASD participants were not scanned because they were uncomfortable in the scanner. The rest of the participants tolerated the scanning procedure well; none of these participants has required any alteration of the MRI or cognitive protocols. Subtask 2 (Analysis of initial baseline data): Initial cross-sectional data analysis of fMRI, cognitive and behavioral data to address Specific Aims 1 and 2 (Evaluate cross-sectional group differences in cognitive functioning and imaging measures). We have completed data analysis on a subset of the cross-sectional data and are currently preparing a manuscript. We are meeting this milestone goal.

- **What was accomplished under these goals?**

Our major activities for the first year includes obtaining all of the regulatory approval to begin the study, recruiting participants and acquiring data, analyzing the preliminary data for significance and publication, presenting our preliminary results at a national meeting for visibility of our study, preparing a manuscript of our preliminary findings, obtaining funding for other related questions in aging and ASD.

Significant results:

Demographic and cognitive results:

We have analyzed cognitive and fMRI data from the first 16 ASD and 17 cognitively normal ("neurotypical" or NT) controls. All ASD diagnoses were confirmed by the trained staff at Southwest Autism Resource and Research Center (SARRC) based on the Autism Diagnostic Observation Schedule. All participants were given the Kaufman Brief Intellectual Test to provide an estimate of intelligence to ensure that all participants were above the IQ= 80 cutoff and to assure general equivalence of IQ between groups. Groups were well-matched according to age, IQ, and education (Table 1). Groups performed similarly on tests of delayed verbal memory (RAVLT) and detailed visual search (Group Embedded Figures Task), but the ASD group made significantly more errors on the executive function task, which requires monitoring,

attention, mental flexibility and concept formation (Wisconsin Card Sorting Test (WCST); Table 1). A significant group difference on WCST errors remained after including demographic variables (age, IQ, or education) as covariates (all $p<0.05$). On fMRI tasks, performance was above 80% on all tasks.

Table 1. Demographic variables, neuropsychological test scores, and fMRI task behavior. Mean (SEM)

	ASD (N=16)	NT (N=17)	T statistic	p value
Age	50.1 (±1.7)	50.1 (±1.7)	0.0	1.0
IQ	108.9 (±3.4)	110.2 (±2.7)	0.31	0.76
Education (Years)	15.0 (±0.6)	15.6 (±0.6)	0.68	0.50
WCST Errors	35.6 (±7.1)	18.7 (±3.5)	2.17	**0.038***
RAVLT Delayed Memory	9.4 (±0.9)	10.1 (±0.8)	0.52	0.60

Functional Connectivity: Differences in Neural Networks using fMRI and DTI

We used independent component analysis (ICA) as a data-driven analysis tool that can identify temporally coherent networks underlying fMRI activity (Calhoun et al., 2001; Kim et al., 2009). ICA was used to evaluate functionally connected neural network activity during the n-back task. The n-back task is a common fMRI task used to interrogate the integrity of networks involved in working memory. The task requires online monitoring and attention to a series of letters. In the version used in this study, participants need to correctly identify targets that range from simple matching a target ("0-back") to identifying matches that are two letters apart (2-back). All participants are pre-trained on this task prior to scanning. All participants performed well (over 80%) on all conditions of the n-back task. The ASD participants' reaction time was slower on the 2-back condition. No other significant differences in performance was observed. Group Independent Components Analysis (ICA) was performed using the Group ICA fMRI Toolbox (GIFT; http://icatb.sourceforge.net). Briefly, the Infomax Algorithm was used to estimate the number of independent components which was determined to be 24. Each component consisted of individual spatial maps and time courses. Group ICA was performed on all the subjects together to ensure the arbitrary order components were the same across each participant (Kim et al., 2009). Spatial maps were averaged across two runs of the n-back task. Each voxel from the spatial maps was calibrated with a z-score denoting the degree to which the time course of each voxel contributed to the average time course of the component (Beckmann et al., 2005). Components were first spatially sorted to standard white matter and cerebral spinal fluid masks, and were disregarded from the analysis if $r^2>0.02$ or $r^2>0.05$,

respectively (Kim et al., 2009). ICA is also able to identify components that result from motion, and visual inspection was done to discard any motion-related component (McKeown et al., 2003). Components were then temporally sorted using the 2-back, 1-back, and 0-back task regressors to assess the degree to which each component was task-modulated (Kim et al., 2009). Unique beta weights were generated for each participant, task condition, and component. One-way ANOVAs were run for ASD and NT groups separately on beta weights for working memory load comparisons (2 vs. 0; 2 vs. 1; 1 vs. 0) and group comparisons were then made on components that were significantly task-related; significance was set at $p<0.001$. Spatial distribution of components was determined by one-way general linear model (GLM) analysis of component maps including all participants in SPM, with a peak voxel threshold of family-wise error (FWE) $p<0.05$.

Results: Task-modulated Components from the n-back task

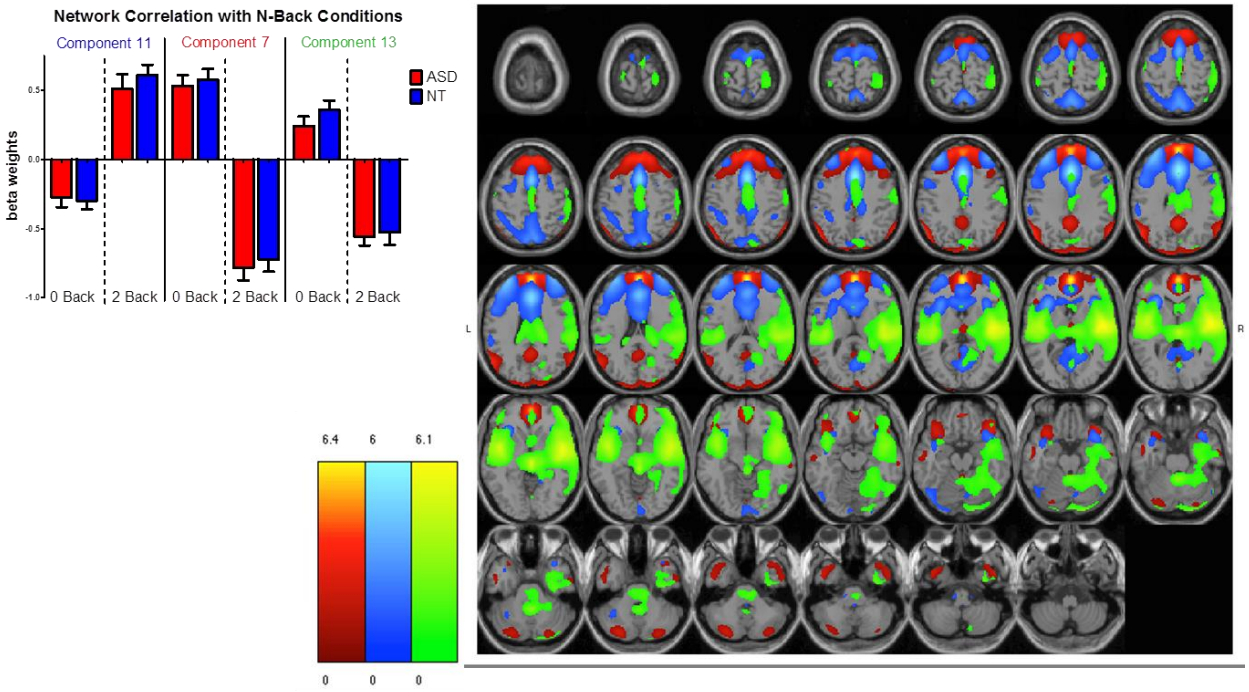

Figure 1. Group differences in network connectivity during a working memory functional MRI task. Average 0-Back and 2-Back beta weights (±SE) per group (left) and spatial maps (right) for each of the three task-modulated components.

To demonstrate the nature of the working memory networks, we first present components showing significant one-way ANOVAs for the 0-back vs. 2-back conditions (i.e., the greatest working memory load) across both the ASD and NT groups (Figure 1). Component 11 was positively related to the 2-back condition and negatively related to the 0-back condition, and comprised a classic cortical working memory network including the bilateral dorsolateral prefrontal cortex (dlPFC), parietal cortex, insula, and the anterior cingulate cortex. Components

7 and 13 were negatively related to the 2-back condition and positively related to the 0-back condition and comprised regions of the Default Mode Network (DMN). Component 7 was driven by the medial PFC (mPFC) but also included the posterior cingulate cortex (PCC) and bilateral inferior parietal cortices. Component 13 was driven by the bilateral temporal cortices but also included the mPFC and PCC. Within this relatively small group, best separation of findings was demonstrated comparing 2 vs. 0 conditions; there were no significant one-way ANOVAs for 0-back vs. 1-back or 1-back vs. 2-back conditions.

ICA network connectivity differences in ASD compared to NT

Component 16 was positively related to the 2-back condition and negatively related to the 0-back condition for the NT group, producing a significant group (ASD vs. NT) by task condition (2-back vs. 0-back) interaction (Fig. 2a below). The beta weights were more positive for the 2-back condition [$F(1,28) = 11.182$; $p = 0.002$] and more negative for the 0-back condition [$F(1,28) = 17.471$; $p < 0.001$] for the NT group (Fig. 2a), while this network was minimally engaged by the ASD group, showing very weak modulation between high and low WM load. This network consisted of the left inferior frontal lobe, bilateral hippocampi, amygdala, striatum, and thalamus (Fig. 2b).

Thus, the older ASD group showed a weak engagement of a subcortical-frontal network commonly engaged during a working memory task, compared to age-matched controls. Striato-thalamo-cortical networks are known to be involved in working memory, impulse control and other aspects of executive functioning (Miller and Cohen, 2001). Recently, Gordon et al. (2015) demonstrated that differences in functional connectivity between regions of this subcortical-frontal network were associated with variability in normal controls. Specifically, lack of engagement of this network resulted in weak performance on the n-back task and increased impulsivity scores within a group of health controls. Our findings from our group differences in engagement of this network along with the greater number of errors produced by the ASD group on the WCST, which requires error monitoring, updating and flexible thinking, suggests that this is a relative weakness within our ASD group, detectible within a small group. We will continue to expand our sample size to ensure that this finding remains stable, as well as begin collecting our second longitudinal data point for those already enrolled in the study.

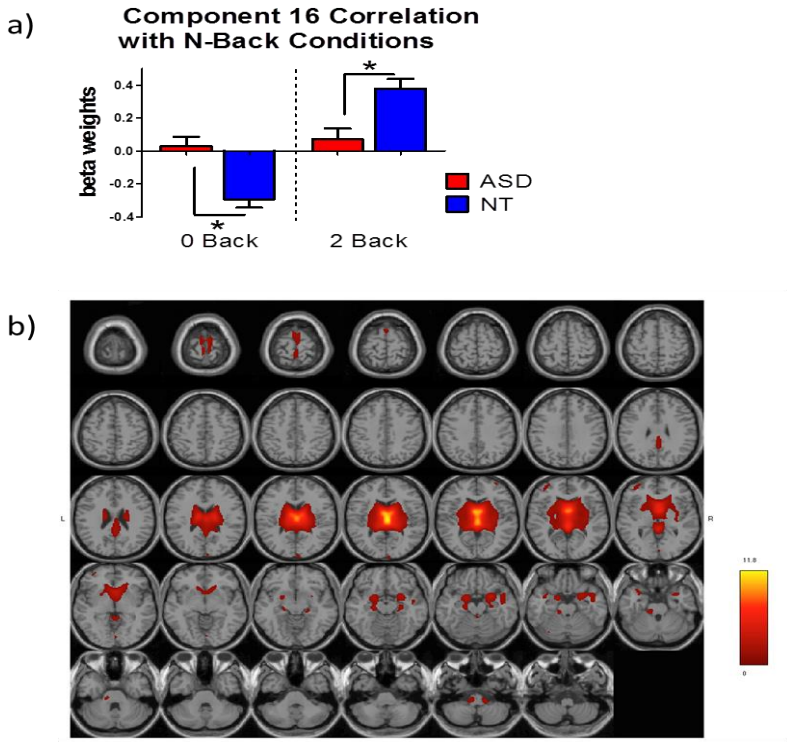

Figure 2. ICA component differences between ASD and NT groups. (a) Average 0-Back and 2-Back beta weights (±SE) per group and (b) spatial maps for Component 16 which was task modulated in the NT but not ASD group. *p<0.001

Structural differences in ASD compared to NT

Using high-resolution 3D T1 weighted and diffusion tensor images allowed us to investigate structural changes related to the connectivity and behavioral differences we have described above.

White matter:

The ASD group showed significantly reduced white matter integrity (voxel-wise differences in Fractional Anisotropy; FA) in the genu of the corpus callosum and bilaterally in the fimbria of the hippocampi. There were no areas of greater FA in the ASD cohort. Decreased CC integrity predicted increased errors on the WCST in the ASD group. No group differences in Mean Diffusivity, another measure of white matter integrity survived correction for multiple comparisons, but consistent with the spatial location of FA differences, two clusters in the bilateral anterior corona radiata (projections from of the genu of the corpus callosum) showed increased MD in the ASD group at p=0.001 uncorrected (not shown).

Brain Volumetric Differences:

Brain volumetric differences were investigated using automatic segmentation in Freesurfer. All volumetric measures were corrected for total intracranial volume. Due to the involvement of the left inferior frontal lobe, genu of the CC, and bilateral hippocampus, thalamus, caudate, putamen, and amygdala in functional and structural connectivity group

differences, these structures were investigated for differences in volume. Bilateral hippocampi and right amygdala were significantly smaller in the ASD group (Table 2).

Table 2. Regional brain volumes in aged ASD and NT

	ASD (N=14)	NT (N=17)	T statistic	p value
L Inferior Frontal Cortex	2.12 (±0.17)	2.20 (±0.13)	1.30	0.21
Genu of CC	0.061 (±0.015)	0.064 (±0.016)	0.59	0.56
L Thalamus	0.52 (±0.10)	0.59 (±0.11)	1.95	0.06
R Thalamus	0.46 (±0.09)	0.52 (±0.10)	1.70	0.10
L Hippocampus	0.31(±0.06)	0.36 (±0.07)	2.18	**0.038***
R Hippocampus	0.31 (±0.05)	0.36 (±0.07)	2.32	**0.028***
L Amygdala	0.12 (±0.02)	0.13 (±0.03)	1.74	0.09
R Amygdala	0.13 (±0.02)	0.15 (±0.03)	2.28	**0.03***
L Caudate	0.23 (±0.05)	0.25 (±0.05)	1.03	0.31
R Caudate	0.24 (±0.05)	0.26 (±0.05)	1.18	0.25
L Putamen	0.38 (±0.07)	0.41 (±0.09)	1.13	0.27
R Putamen	0.37 (±0.07)	0.40 (±0.09)	1.30	0.20

Other related accomplishments:

Received funding for a study of Emotion in Autism:

We received a grant from the Institute for Mental Health Research to study emotional aspects of aging and ASD. The literature indicates that ASD individuals have greater rates of comorbid anxiety and depression. Our assessment includes self-report measures of anxiety and depression self-report at the time of cognitive testing and MRI scanning. Data from the baseline assessment of some of our participants showed that 88% of the middle-age ASD group reported significant levels of anxiety and 44% reported significant depression, as compared to 45% of a group of 8 young-adult ASD for both anxiety and depression. Social network measures did not significantly correlate with mood measures in either middle-age or young-adult ASD, and the report of caregivers was not correlated with the symptom severity reported by the participants. Participants from our group of TDs are not part of these analyses since our exclusion criteria for TDs includes current or past psychiatric illness or symptoms. Interestingly, anxiety and depression symptoms correlated with several cognitive measures for the young ASD group, but there was no correlation in the older ASD group with cognition. This suggests that the cognitive deficits observed in the older ASD participants are not due to the presence of anxiety and depression but instead anxiety and depression may independently be affected in aging. These findings were presented at the International Meeting for Autism Research in May 2016. Furthermore, we

received extramural funding to further investigate emotional status in our ASD cohorts. In this study, we recruit the same participants who contributing to the MRI/cognition study. Participants undergo a clinical interview by a psychiatrist and are assessed using the Structured Clinical Interview for DSM Disorders (SCID for DSM-IV) to better understand how individuals with ASD express/self-report anxiety and depression.

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Kim DI, Mathalon Dignity Health, Ford JM, Mannell M, Turner JA, Brown GG, Belger A, Gollub R, Lauriello J, Wible C, O'Leary D, Lim K, Toga A, Potkin SG, Birn F, Calhoun VD. Auditory oddball deficits in Schizophrenia: An independent component analysis of the fMRI multisite function BIRN study. Schizophr Bull 2009;35(1): 67-81.

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Miller EK, Cohen JD. 2001. An integrative theory of prefrontal cortex function. Annu Rev Neurosci. 24:167.

- **What opportunities for training and professional development has the project provided?**

  Training and professional development was not a major goal of this project; however, this project provided the environment to train and promote Dr. Baxter's post-doctoral resident, who is one of the key personnel in this study. B. Blair Braden, PhD has been active in all of our projects on ASD and aging. She came to the lab after graduate training in neuroscience, studying hormone and aging in animal models specifically to help develop our cognitive and imaging studies in aging and Autism. She is transitioning to a tenure-track Assistant Professor position at Arizona State University and establishing a lab in Autism and Aging. We will continue to collaborate on this project together; we petitioned for, and we were granted permission to transition Dr. Braden's support from this grant to ASU for her continued participation on the project during Year 2 of funding.

- **How were the results disseminated to communities of interest?**

Autism Brain Imaging Data Exchange: The Autism Brain Imaging Data Exchange II is large-scale data repository of ASD and TD controls from 17 sites. Our contribution of 58 samples (which includes participants that are part of the DoD study) include the oldest sample in the group (aged 64 years old) and represents the first substantial set of older adults in the exchange. Our contribution to this high-profile, international group of researchers helps us to disseminate our study to the ASD research community.

- ***Aging in Autism Special Interest Group, International Society for Autism Research (INSAR).*** Both Drs. Baxter and Braden are members of the recently-formed group of Autism researchers dedicated to the study of older individuals with Autism. The group was formed by Hilde Geurts, PhD of the University of Amsterdam to work towards common goals for studying older adults with ASD. Both Drs. Baxter and Braden are part of the founding members and will contribute to the establishment of a core set of cognitive tests and other data that can be collated across studies from around the world.

National Media Attention for the Project

SARRC and BNI were approached by Ivanhoe Broadcast News for a story regarding our unique study. The final product can be viewed from this link. http://abc7.com/health/adult-men-with-autism-participate-in-one-of-a-kind-study/1429782/ This report has received much attention both from the press; the story has aired in at least 8 different states in July and August, reaching markets as large as Chicago and Los Angeles, with an estimated 1.8M impressions.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Continue with recruitment of ASD and age-matched TDs to reach our target sample of 35/group.
  - Begin second time point evaluations of those participants who are two years from their original study.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

We have significantly increased the number of older adults to a national data exchange. Our study is complementing the established cognitive studies in older individuals with Autism to emphasize the importance of developing a greater understanding of aging in Autism to inform treatment.

What was the impact on other disciplines?

Nothing to Report.

- **What was the impact on technology transfer?**

  Nothing to Report.

What was the impact on society beyond science and technology?

- An intent of our study is to develop a plan of action to help keep older adults with ASD as independent as possible for as long as possible. We foresee that the results of our study, which is one of the first of its kind, will be able to inform state agencies and community aging programs to develop interventions that will help keep older ASDs independent. We plan on publishing our results, and becoming a voice for the older ASD population, to help form effective and meaningful supports and treatments for this group.

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

   - **Changes in approach and reasons for change**

     Nothing to Report

   - **Actual or anticipated problems or delays and actions or plans to resolve them**

   Obtaining institutional and HRPO approval for the study took longer than expected, so recruitment of our cohort is somewhat behind schedule. Our goals are to use all available resources to recruit our target number as soon as possible.

   - **Changes that had a significant impact on expenditures**

   Our expenditures for MRI scans and patient reimbursement is less than expected over the first year because of the delays in obtaining institutional approval, and patient recruitment.

   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

- We changed our local consent and HIPAA forms to allow us to share anonymized data with the Autism Brain Imaging Date Exchange (detailed above). This was approved by

our institution. We informed the HRPO, who also approved this. Current approval dates are: Informed Consent: 4/20/16; HIPAA: 3/02/16

- **Significant changes in use or care of human subjects:** Nothing to Report
- **Significant changes in use or care of vertebrate animals. N/A**
- **Significant changes in use of biohazards and/or select agents N/A**

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Nothing to Report

- **Journal publications.** Nothing to Report
- **Books or other non-periodical, one-time publications.** Nothing to Report
- **Other publications, conference papers, and presentations.** Nothing to Report
- **Website(s) or other Internet site(s)**
  Website/link to our media coverage: http://abc7.com/health/adult-men-with-autism-participate-in-one-of-a-kind-study/1429782/
- ***List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided.*** Nothing to report
- **Technologies or techniques**
  None to report
- **Inventions, patent applications, and/or licenses**
  None to report
- **Other Products**
  None to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	*Leslie C. Baxter, PhD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-3971-863X*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Baxter oversaw data collection, analysis and interpretation*
Funding Support:	National Institute on Aging NIA 5 P30 AG019610-03 (Reiman, PI; Baxter, Site PI) State of Arizona, Arizona Alzheimer's Consortium

Name:	*Brittany Blair Braden, PhD*
Project Role:	Post-doc
Researcher Identifier (e.g. ORCID ID):	0000-0001-6842-9784
Nearest person month worked:	6
Contribution to Project:	*Dr. Braden scheduled, assessed, scanned patients and analyzed data.*
Funding Support:	State of Arizona, Arizona Alzheimer's Consortium

- **Has there been a change in the active other support of the PD/PI(s) or**
- **senior/key personnel since the last reporting period?**

- Nothing to Report during Year 1.
- **What other organizations were involved as partners?**
- **Organization Name**: Southwest Autism Resource and Research Center
- **Location of Organization:** 2225 N 16th Street Phoenix, AZ 85006
- **Partner's contribution to the projec**t Partnering PI
- **Collaboration** Recruitment of participants, collaboration with data interpretation and manuscript preparation